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Efficiency of Three Diets for Larval Development in Mass Rearing *Aedes albopictus* (Diptera: Culicidae)

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ABSTRACT A fundamental step in establishing a mass production system is the development of a larval diet that promotes high adult performance at a reasonable cost. To identify a suitable larval diet for *Aedes albopictus* (Skuse), three diets were compared: a standard laboratory diet used at the Centro Agricoltura Ambiente, Italy (CAA) and two diets developed specifically for mosquito mass rearing at the FAO/IAEA Laboratory, Austria. The two IAEA diets, without affecting survival to the pupal stage, resulted in a shorter time to pupation and to emergence when compared with the CAA diet. At 24 h from pupation onset, 50 and 90% of the male pupae produced on the CAA and IAEA diets, respectively, had formed and could be collected. The diet received during the larval stage affected the longevity of adult males with access to water only, with best results observed when using the CAA larval diet. However, similar longevity among diet treatments was observed when males were supplied with sucrose solution. No differences were observed in the effects of larval diet on adult male size or female fecundity and fertility. Considering these results, along with the relative costs of the three diets, the IAEA 2 diet is found to be the preferred choice for mass rearing of *Aedes albopictus*, particularly if a sugar meal can be given to adult males before release, to ensure their teneral reserves are sufficient for survival, dispersal, and mating in the field.

KEY WORDS *Aedes albopictus*, larval diet, mass rearing

Aedes albopictus (Skuse), indigenous to South East Asia and islands of the Western Pacific and Indian Ocean, has over the past two decades been one of the fastest spreading animal species. It has expanded, mainly through the international trade in used tires, from its native range to all continents including North, Central, and South America, the Middle East, Africa, Europe, and Australasia (Reiter and Sprenger 1987, Benedict et al. 2007, Scholte and Schaffner 2007). This dispersion raises concerns not only because of the high anthropophily and nuisance biting aspects of the species' diurnal behavior but primarily its confirmed vector competence for several arboviruses including dengue and chikungunya (Gratz 2004) and also its possible involvement in the Usutu virus (USUV) transmission cycle (Calzolari et al. 2010).

The first record of *Ae. albopictus* being found in Italy dates from 1990 and, despite attempts to block its spread, this species is now present throughout Italy (Sabatini et al. 1990, Scholte and Schaffner 2007, European Centre for Disease Prevention and Control 2009). Until now conventional pest control methods have failed to keep this species below sustainable and safe levels (Carrieri et al. 2006), probably because of the peculiar eco-biology of *Ae. Albopictus*—it exploits a variety of water-collecting containers found in private gardens, backyards, and urban vegetated areas (Bellini et al. 2010). Until the summer of 2007, *Ae. albopictus* was merely a nuisance in Italy, but it went on to cause the first outbreak of chikungunya in Europe (Romi et al. 2008).

Since 1999, the Centro Agricoltura Ambiente (CAA) "G. Nicoli" in Crevalcore, Bologna (Italy), has been conducting research to measure the application of the sterile insect technique (SIT) together with other existing control methods to suppress *Ae. albopictus* (Bellini et al. 2007). The SIT is a biological method of controlling insect pests using sustained releases of large numbers of mass-reared and sexually sterilized males to reduce the fertility of a field population of the same species. Effective control is achieved when the sterile insects are used systematically as part of area-wide integrated pest management (AW-IPM) programs (Knipling 1955, Coleman and Alphey 2004, Hendrichs and Robinson 2009). Application of the SIT involves the colonization of the target species and the large-scale rearing of viable and competitive males for release, which must be able to locate and mate with wild females. Therefore, the colonization and produc-

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tion processes must preserve and, if possible, enhance the characters necessary for these functions (Benedict et al. 2009b). In the mass rearing phase, larval diet quality and rearing conditions have a direct and often irreversible effect on adult traits (Briegel and Timmermann 2001, Cohen 2003, Benedict et al. 2009b).

As reported by Timmermann and Briegel (1999), the larval diet should provide a wide range of nutrients to avoid the risk of deficiencies that could negatively affect both the rearing productivity and the fitness of the males produced.

Another aspect that has to be considered when selecting a diet for mass rearing purposes is the choice of the components. As reported by Cohen (2001), ingredients such as wheat germ, soy flour, ground beef, and chicken eggs are relatively inexpensive and many are highly nutritious; it would be more expensive and logistically challenging to rear many successive generations of fecund insects on a chemically defined diet. In addition, such diets would be very expensive—either in cost per kilogram or in preparation time.

The objective of this study was to compare three diets used for the larval development of *Ae. albopictus*, seeking to find which was the best for mass rearing purposes.

Materials and Methods

Mosquitoes. The *Ae. albopictus* strain used for all the experiments was collected as eggs from field sites in Rimini (Italy). The colony was kept for five generations in a climate-controlled room at 28 ± 2°C, 80 ± 5% relative humidity (RH), and a photoperiod of 14:10 (L:D) h. The rearing methods used were the same as those described in Balestrino et al. (2010).

Diets. One diet developed at the CAA (CAA diet) and two diets developed at the FAO/IAEA (IAEA 1 and IAEA 2 diets) were compared. For all diets, a 4% wt:vol slurry of each diet was prepared by mixing the solid components, consisting of small particles, by hand in deionized water, and 10 ml aliquots were stored at −20°C to prevent the proliferation of microorganisms and degradation of the diet.

The composition of the three diets was as follows: CAA—80% Friskies dry adult cat food (Nestlé S.A., Vevey, Switzerland), 14% brewer's yeast (Sigma Aldrich Inc., St. Louis, MO), and 6% Tetramin fish food (Tetra Pro, Melle, Germany) (Bellini et al. 2007); IAEA 1—50% bovine liver powder (MP Biomedicals, Santa Ana, CA), 50% tuna meal (T. C. Union Agrotech, Thailand), and, as an additive, 0.4% wt:vol of Vitamin Mix (Vanderzant Vitamin Mix, Bio-Serv, Frenchtown, NJ) based on the diet used by Damiens et al. 2012; and IAEA 2—25% bovine liver powder, 50% tuna meal, 12.5% brewer's yeast (MP Biomedicals), 12.5% squid powder (T. C. Union Agrotech), and 0.4% wt:vol of Vitamin Mix.

Effects of Diets on Larval Development. To measure the effects of the diets on *Ae. albopictus* larval development, 750 first instar larvae (L1) were manually counted and added to each of three transparent plastic containers (16.6 by 16.6 by 8 cm). Each container was filled with 500 ml of deionized water so as to obtain a larval density of 1.5 larvae per ml and a water depth of ≈2 cm. Every day an aliquot of 10 ml (corresponding to 0.53 mg per larva) of each diet, as determined from previous work (J.R.L.G., unpublished data), was defrosted and placed in the corresponding container until all the larvae pupated. On day five posthatching, a double amount of diet was placed in each tray to provide for the increased feeding requirements, as larvae neared pupation (Timmermann and Briegel 1999, Medici et al. 2011). All containers were checked daily at 0900, 1200, and 1500 hours, and any pupae that had formed were manually collected. The pupae were counted and placed in transparent plastic tubes (5 cm in diameter, 10 cm in height). Each tube was filled with 50 ml of deionized water and covered with a sponge plug. Adult emergence was also recorded daily at 0900, 1200, and 1500 hours, and sex was determined. The experiment was repeated three times for each diet.

Time to pupation and time to emergence were calculated as the development duration between L1 and pupa and L1 and adult stages, respectively. Survival to pupation and survival to adult emergence were calculated as the proportion of larvae that survived from L1 to the pupal stage and from L1 to the adult stage, respectively.

In mass rearing, the protandry and smaller size of male compared with female pupae can be exploited by collecting the pupae formed in the first 24 h of pupation, which are majority male, and mechanically sieved for sex separation (Bellini et al. 2007). Male pupae production rate at 24 h was defined as the number of male pupae formed and manually collected in the first 24 h (from the beginning of pupation) divided by the total number of male pupae collected from each diet treatment. The sex ratio was calculated as the proportion of all male to female adults emerging from a treatment, either for the adults that emerged from pupae produced in the first 24 h from pupation onset (sex ratio at 24 h) and for the total number that emerged (overall sex ratio).

Adult Male Longevity and Size. Fifty males (<24 h old) that had emerged from each diet treatment were placed in separate plastic cages (30 by 30 by 30 cm) (BugDorm 1; MegaView, Taichung, Taiwan), with unlimited access to a sugar solution (10% sucrose, 0.2% methylparaben) (Benedict et al. 2009a) or to water only to measure the longevity of the males when caged with other males—in Table 2 as M [M]. Three cages with free access to sugar and three cages with access only to water were set up for each diet treatment. Mortality was recorded every day at 0900 hours by removing and counting the dead individuals from each cage, which were stored at −20°C and used for wing-length measurements.

The right wing (or left if the right was damaged or lost) was removed under a dissecting microscope from a sample of 28 individuals from each treatment. A wing was measured from the distal edge of the alula to the end of the radius vein excluding fringe scales (Packer

Table 1. Development parameters (mean ± SE) of *Aedes albopictus* fed on larval diets CAA, IAEA 1, or IAEA 2

Parameter	Stage	Sex[a]	CAA	IAEA 1	IAEA 2
Development time (d from L1)	Pupa	M	5.53 ± 0.09a	4.89 ± 0.03b	4.88 ± 0.03b
	Pupa	F	6.37 ± 0.05a	5.61 ± 0.03b	5.70 ± 0.07b
	Adult	M	7.55 ± 0.10a	6.83 ± 0.03b	6.79 ± 0.01b
	Adult	F	8.54 ± 0.06a	7.69 ± 0.04b	7.80 ± 0.06b
Survival rate (on L1)	Pupa	M + F	0.968 ± 0.023a	0.891 ± 0.033a	0.926 ± 0.011a
	Adult	M + F	0.886 ± 0.048a	0.700 ± 0.002b	0.809 ± 0.020ab
	Adult	M	0.429 ± 0.032a	0.377 ± 0.020a	0.412 ± 0.009a
	Adult	F	0.456 ± 0.018a	0.324 ± 0.018b	0.397 ± 0.018ab
Male pupae production rate at 24 h[b]	Pupa	M	0.50 ± 0.06a	0.91 ± 0.01b	0.90 ± 0.00b
Sex ratio at 24 h	Pupa	M/F	4.72 ± 0.01a	2.36 ± 0.31b	2.42 ± 0.10b
Sex ratio	Adult	M/F	0.94 ± 0.08a	1.18 ± 0.13a	1.04 ± 0.06a

Means (based on three replicates) within a row followed by the same letter are not significantly different ($P = 0.05$; Tukey's mean separation test).

[a] M + F, both males and females; M and F, males and females, respectively; and M/F, sex ratio.

[b] Number of male pupae obtained in the first 24 h (from the beginning of pupation) divided by the total number of male pupae produced for each diet treatment.

and Corbet 1989). A digital image of the wing was made using a CC-12 camera mounted on a stereomicroscope, and measurements were performed with analySIS B software (Olympus Soft Imaging Solutions GmbH, Münster, Germany).

Adult Longevity and Egg Production. Males (n = 100) and females (n = 100) (<24 h old) that had emerged from each diet treatment were placed together in plastic cages with a sugar solution as described above. After 5 d, and for the following 14 d, a bloodmeal consisting of fresh mechanically defibrinated bovine blood was offered daily to the females through a heated metal plate, covered with stretched Parafilm M (Pechiney Plastic Packaging Company, Chicago, IL), and placed externally, on the netted bottom of the cages. Two days after the first bloodmeal, a plastic 250-ml beaker containing 100 ml deionized water and a strip of white filter paper (white creped papers IF C140, Industrial Filtro S.r.l., Cologno Monzese, Italy) was placed inside each cage to provide an oviposition surface. Each day (until the last oviposition) the filter papers with eggs were removed and replaced with new papers. To measure the longevity of the males and the females when caged together—indicated respectively as M [F] and F [M] in Table 2—every day the mortality was monitored by removing the dead individuals from the cages and recording the number of each sex removed.

After removal from the cages, the filter papers were left to dry inside a climate-controlled room for 24 h, during which time complete embryonation occurred. Then the number of eggs on the filter papers was counted under a stereomicroscope.

From the first day of oviposition onwards, samples of filter papers with oviposited eggs were collected every other day. Eggs were hatched according to the method of Balestrino et al. 2010 and fertility measured for each treatment by observing the percentage egg hatch. Each treatment was replicated three times.

Statistical Analysis. All statistical analyses were performed using MiniTab (MiniTab Inc., State College, PA).

The effects of the three diets on survival to pupation, survival to adult emergence, and male pupae production were analyzed using the General Linear model (GLM) after angular (arcsine sqrt) transformation of the data expressed in percent. Data on the time to pupation, time to emergence, and sex ratio were analyzed using the GLM. Means were separated by Tukey's mean separation test ($P < 0.05$).

The Kaplan–Meier method was used to estimate the mean male adult life span under the various larval diet and adult feeding regimes. Longevity differences among the treatments were compared using Log-rank test. The effect of diet on male wing length was measured using GLM. Data on egg production and fertility were analyzed using GLM after angular (arcsine sqrt) transformation of the data expressed as percentages. Means were separated by Tukey's mean separation test ($P < 0.05$).

Results

Larval Development. Time to pupation and time to emergence differed significantly between the diets ($F_{2, 12} = 118.1$; $P < 0.001$ and $F_{2, 12} = 119$; $P < 0.001$, respectively). Larvae reared on CAA diet took a longer time to pupate and to emerge as adults compared with larvae fed on IAEA 1 ($t = -13.7$ and -13.63; $P < 0.001$) and IAEA 2 ($t = -12.8$ and -13.07; $P < 0.001$) diets, but no differences in time to pupation and emergence were observed between diets IAEA 1 and IAEA 2 ($t = 0.847$ and 0.843; $P > 0.05$) (Table 1). For all diets, males pupated and emerged faster than females ($F_{1, 12} = 359.8$ and 409.4; $P < 0.001$) (Table 1).

Survival to pupation did not differ significantly among diets ($F_{2, 6} = 2.66$; $P > 0.05$), but survival to adult emergence was significantly affected ($F_{2, 12} = 10.87$; $P < 0.05$) (Table 1). Larvae fed on the CAA diet showed a significantly higher survival to adult emergence compared with the IAEA 1 diet ($t = -4.347$; $P < 0.05$) but not in comparison with larvae reared on the IAEA 2 diet ($t = -1.790$; $P > 0.05$). No significant difference was observed in survival to adult emergence between IAEA 1 and IAEA 2 diets ($t = 2.557$; $P > 0.05$) (Table 1). The survival to adult emergence analysis performed as a function of sex showed statis-

Table 2. Longevity (d) (mean ± SE) of adults developed from larvae fed on diets CAA, IAEA 1, or IAEA 2

Adult food	Sex[a]	CAA	IAEA 1	IAEA 2
Water	M [M]	5.17 ± 0.08a	4.84 ± 0.06b	4.49 ± 0.07c
Sugar	M [M]	30.17 ± 1.75a	31.46 ± 1.19a	32.58 ± 1.22a
Sugar	M [F]	14.31 ± 0.52a	10.15 ± 0.43b	13.81 ± 0.58a
Sugar	F [M]	18.92 ± 0.77a	17.33 ± 0.63a	19.29 ± 0.63a

Means (based on three replicates) within a row followed by the same letter are not significantly different ($P = 0.05$; Tukey's mean separation test).

[a] M [M], longevity of males when caged with other males; M [F], longevity of males when caged with females; F [M], longevity of females when caged with males.

tical differences among females fed on different diets ($F_{2, 6} = 14.15$; $P < 0.01$) but not among males ($F_{2, 6} = 1.46$; $P > 0.05$) (Table 1).

For 24 h after the beginning of pupation, the male pupae production rate was significantly different among the diets ($F_{2, 6} = 55.26$; $P < 0.001$). The proportion of males obtained using the CAA diet was significantly lower than that observed using IAEA 1 ($t = 9.238$; $P < 0.001$) and IAEA 2 ($t = 8.964$; $P < 0.001$) diets. The two IAEA diets yielded a similar male production ($t = -0.274$; $P > 0.05$) (Table 1). The sex ratio of the adults produced in the first 24 h of pupation (sex ratio at 24 h) was significantly different between diet treatments ($F_{2, 6} = 51.48$; $P < 0.001$). The proportion of males from these pupae on the CAA diet was significantly higher than that observed on the IAEA 1 ($t = -8.88$; $P < 0.001$) and IAEA 2 ($t = -8.69$; $P < 0.001$) diets, but no difference was observed between IAEA 1 and IAEA 2 diets ($t = 0.197$; $P > 0.05$, Table 1). The overall sex ratio observed was not significantly different between the three diet treatments ($F_{2, 6} = 1.6$; $P > 0.05$) (Table 1).

Adult Male Longevity and Size. When male adults had access to water only, longevity significantly differed between diet treatments (χ^2 (2) = 65.11; $P < 0.001$) (Table 2—water; M [M]). Males reared on the CAA diet had a higher longevity compared with males fed on the IAEA 1 (χ^2 (1) = 18.85; $P < 0.001$) and IAEA 2 diets (χ^2 (1) = 51.57; $P < 0.001$). Males reared on IAEA 1 lived longer than males reared on IAEA 2 (χ^2 (1) = 22.23; $P < 0.001$) (Table 2—water; M [M]).

When males were caged with free access to a sugar solution, their longevity was ≈30 d and did not differ among the diets (χ^2 (2) = 3.17; $P > 0.05$) (Table 2—sugar; M [M]).

No difference in the wing length of males reared on the three diets ($F_{2, 81} = 0.05$; $P > 0.05$) was observed. The mean wing lengths (±SE) were 2,170 (9), 2,173 (14), and 2,179 μm (28) for the CAA, IAEA 1, and IAEA 2 diets, respectively.

Adult Longevity and Egg Production. When males were caged with females and given unlimited access to sugar, male longevity was significantly different between diet treatments (χ^2 (2) = 42.54; $P < 0.001$) (Table 2—sugar; M [F]), but no difference in female longevity was observed (χ^2 (2) = 5.2; $P > 0.05$) (Table 2—sugar; F [M]). The longevity of males fed on CAA and IAEA 2 diets was significantly longer than that of males fed on the IAEA 1 diet (χ^2 (1) = 34.12; $P < 0.001$; χ^2 (1) = 24.76; $P < 0.001$), and no difference in longevity was observed between males fed on CAA and IAEA 2 diets (χ^2 (1) = 0.25; $P > 0.05$) (Table 2—sugar; M [F]). When given access to sugar, males caged without females lived significantly longer than males caged with females ($F_{1, 12} = 397.8$; $P < 0.001$) regardless of the diet during the larval stages ($F_{2, 12} = 2.11$; $P > 0.05$).

Egg production per cage as a function of the different diets did not differ between the treatments ($F_{2, 6} = 2.68$; $P > 0.05$). The mean (±SE) egg production per cage was 3,345 (±231), 3,835 (±404), and 4,484 (±385) for adults reared on CAA, IAEA 1, and IAEA 2 diets, respectively. No statistical differences were observed in daily oviposition between different larval diet treatments (all daily comparisons $P > 0.05$). Also, there was no difference in the hatching rate among the three diet treatments ($F_{2, 6} = 2.78$; $P > 0.05$). The mean hatching rate (±SE) was 0.80 (±0.01), 0.86 (±0.03), and 0.88 (±0.04) for treatments reared on CAA, IAEA 1, and IAEA 2 diets, respectively.

Discussion

The CAA diet represents an evolution of a classical laboratory diet for mosquitoes with subsequent enrichment of nutritional components; the main purpose was to provide a wide range of nutrients to the immature stages. Initially composed of finely crushed dry cat biscuits, new components such as brewer's yeast and fish food (Tetramin) were introduced, which increased survival of early larval stages and pupal productivity, respectively (Bellini et al. 2007). In contrast, the IAEA diets were specifically designed to be used for mass rearing, seeking to provide adequate nutritional components to optimize rearing and adult insect quality from ingredients that are inexpensive, globally available, and of consistent quality (Parker 2005, Benedict et al. 2009b). The first version of the IAEA diet (IAEA 1) developed at the Insect Pest Control Laboratory of the IAEA (Damiens et al. 2012) was later revised (to produce the IAEA 2 diet) to reduce costs and broaden the nutritional spectrum of the diet through the introduction of brewer's yeast powder and squid liver powder and the halving of the amount of beef liver powder (D. D., personal observations).

Optimization of *Ae. albopictus* mass production requires the development of a larval rearing module that gives high larval survivorship, fast and homogenous larval development, size homogeneity within the population, synchronicity of the onset of pupation and produces high-quality adults in terms of longevity, flight ability, mating capacity, fecundity, and fertility (Bellini et al. 2007, Medici et al. 2011).

Feeding larvae with IAEA 1 and IAEA 2 diets shortened the time to pupation and to adult emergence compared with larvae fed on the CAA diet. Larvae reared with the two IAEA diets probably reached their critical weight earlier than those reared with CAA diet because of the diet composition. As reported by

Chambers and Klowden (1990), nutritional reserves play a regulatory role in insect development influencing the ability of larvae to pupate. Assuming an equal diet requirement, a shorter time to pupation would produce a shorter rearing schedule for obtaining the males to be released and thus a reduced operational cost of mass rearing.

Variation in the survival to adult emergence rate between diet treatments was only observed in females, while the same proportion of males reared on the various larval diets survived to the adult stage. In the IAEA 1 diet, there could be a lack of nutrients, which is detrimental to the development of females but not to that of males. As reported by Chambers and Klowden (1990), the accumulations of both carbohydrates and lipids at immature stage are sex dependent in *Aedes aegypti.* This putative nutritional deficit appears to be reduced in the IAEA 2 diet, suggesting that the additional components added to this diet partially compensate for this deficiency. Considering the similarity between squid and bovine liver powders in terms of protein composition (Damiens et al. 2012), it is likely that brewer's yeast is responsible for improving the effectiveness of this diet.

On emergence, mosquitoes have teneral reserves of carbohydrates (glycogen) and lipids (triglyceride) accumulated in the larval stage, which are used for adult survival (Van Handel 1965, , Nayar 1968) and flight (Clements 1955, Nayar and Van Handel 1971). An analysis of the diet ingredients showed that squid liver powder, tuna meal, and bovine liver powder are rich in proteins, vitamins, and fatty acids (Damiens et al. 2012), while in brewer's yeast, among other components, there are also carbohydrates (Nestel and Nemny–Lavy 2008), which in *Ae. aegypti* were found to be required for optimal growth and development (Sneller and Dadd 1977). Besides brewer's yeast, the CAA diet contains several other sources of carbohydrates (according to labels, dry cat and fish food both contain cereals, yeasts, and sugars), which could provide adequate glycogen teneral reserves for the production of competitive adult males for release. When tested singularly brewer's yeast did not produce good results in terms of survival to pupation (Damiens et al. 2012); however, when added to the larval diet of *Ae. aegypti*, development time was shortened by 2 d (Akov 1962).

The male pupae production rate at 24 h was higher in treatments fed on the two IAEA diets compared with those given the CAA diet. At 24 h from the onset of pupation, the CAA treatments showed a male-biased sex ratio in comparison with IAEA diets where more female pupae were produced. Further study on pupal size is necessary to determine whether the increased number of females produced by feeding with the IAEA diets could affect the number of females accidentally collected with the males during size-based separation and therefore potentially released. In mass rearing of *Aedes* mosquitoes, it is important to obtain most of the pupae at the same time to maximize the efficiency of the mechanical sex separation procedure, which takes place at 24 h after the onset of pupation. The collection of ~90% of the total male pupal production in the first 24 h from pupation observed when feeding with the IAEA diets could bring great cost effectiveness to mass production. Synchronizing pupation time also allows more accurate determination of pupal age, which helps to minimize the negative effects of the irradiation procedures (Balestrino et al. 2010). Further investigation could also be done into a diet component able to enhance the natural protandry of this species.

Male longevity was only significantly shorter in adults reared on the IAEA diets compared with those reared on the CAA diet when males were kept without access to sucrose solution. The increased longevity of males reared on the CAA diet could be because of the prolonged phagoperiod. As reported by Briegel and Timmermann (2001), the longer the larval feeding period the more reserves can be synthesized, leading to an exponential lipid accumulation in newly emerged males; increased levels of lipids and glycogen are beneficial to the survival of males. In *Ae. Aegypti*, Van Handel (1988) observed that carbohydrate assimilation reached its maximal levels at late fourth instar stage just before pupation, reinforcing the hypothesis that a prolonged larval period can result in an increased level of glycogen reserves.

These results suggest a lack of carbohydrates in the IAEA diets, which can be compensated for by an intake of sugar at the early adult stage, as demonstrated by the fact that the longevity of males, when caged with other males and with free access to a sugar solution, did not differ between treatments. The longevity of males, when caged with females, was shorter than that of males caged alone for all the diet treatments, as also reported by Liles and De Long (1960). However, in contrast to adults obtained from larvae fed on the IAEA 2 and CAA diets, the males reared on IAEA 1 diet and nourished with sucrose solution suffered an increased mortality. This would suggest that 1) the integration of sucrose solution is not sufficient or adequate to support the metabolic energy demand for functions greater than the basal metabolic rate, and 2) brewer's yeast in the diet (absent in IAEA 1 diet) increases metabolic reserves to support these functions.

Van Handel (1988) observed that newly emerged adults from pupae with low glycogen reserves would have to find a food source sooner than those with adequate level of carbohydrates accumulated at immature stages. Further research is necessary to test the effect of larval diets enriched with carbohydrates on parameters related to the adult male fitness. The effect of the administration of sucrose solution to newly emerged males after release has been also measured and shown to have a great impact during harsh climatic conditions (R.B., unpublished data).

An optimum diet for mass rearing should be inexpensive and of a defined quality and composition. Even if, for some mosquito species, diets including components present in the natural breeding environment, such as organic matter and grass cuts, are more efficient for larval rearing than those consisting of

refined components, the former have the disadvantage of not being practical for mass rearing—their acquisition and administration are more difficult when maintaining large colonies, they are less homogeneous, and a huge amount of material must be processed (Sy and Campos 2008). The approximate cost per killogram of the diets is as follows: 32 USD for IAEA 1, 18 USD for IAEA 2, and 33 USD for CAA. Considering cost, and also the fact that no difference was observed in the size of males or female fecundity and fertility in adults reared on the different diets, this preliminary study shows that the IAEA diet integrated with brewer's yeast, IAEA 2, is preferred over the CAA diet for *Ae. albopictus* mass rearing, even if further investigation will be necessary to find a supplement to the diet that overcomes the lack of carbohydrates in the diet, either in terms of quality and quantity.

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